Remarks

The Notice to File Missing Parts of Application indicates that the oath or declaration is unsigned. Applicants attach hereto a copy of the Notice as **Exhibit A**. In response, applicants submit as **Exhibit B** hereto a signed Declaration and Power of Attorney pursuant to 37 C.F.R. §1.53(f). In compliance with 37 C.F.R. §1.63, the Declaration refers to the application's above-identified serial number and filing date.

The surcharge under 37 C.F.R. §1.16(e) for responding to the Notice to File Missing Parts of Application is SIXTY FIVE DOLLARS ($65.00) for a small entity. A check for a total amount of $65.00 is enclosed to cover the surcharge for filing the enclosed Declaration and Power of Attorney.

Furthermore, in compliance with the April 19, 2002 Notice, applicants respectfully comply with the Examiner's Notice and submit a Sequence Listing attached hereto as **Exhibit C** in compliance with the requirements of §1.821-1.825. In addition, applicants submit herewith the Sequence Listing on the enclosed computer diskette. Moreover, applicants submit as **Exhibit D** a Statement In Accordance With 37 C.F.R. §1.821(f) certifying that the computer readable form and paper copy are the same.

Applicants have also amended the subject specification to include the Sequence Id. identifiers where appropriate.
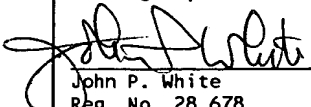
If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant' s undersigned attorney invites the Examiner to telephone the number provided below.

No fee, other than the enclosed $65.00 surcharge fee, is deemed necessary in connection with the filing of this Communication. If any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Attorney for Applicant
Cooper & Dunham LLP
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New York, New York 10036
(212) 278-0400

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents Washington, D.C. 20231

John P. White   5/31/02
Reg. No. 28,678   Date

substrate recognition sequence. A number of SNase constructs have been engineered that contain the MTG substrate recognition sequence in the exposed loop. Based on these, genes that code for receptor fusion constructs have also been constructed.

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Based on the published data, and especially reports from Ajinomoto (4), four substrate recognition sequences were constructed into a biologically inert version of SNase. The four sequences are: i) LGQG   (SEQ. ID. NO.: 1)

10   ii) LQGG   (SEQ. ID. NO.: 2)
           iii) LLQG   (SEQ. ID. NO.: 3)
           iv) LGGG   (SEQ. ID. NO.: 4)

The first three sequences are substrates for TG modification; the forth sequence is a control sequence that is not recognized

15 nor modified by the TG. All four constructs have been made and transformed into *E. coli*; frozen stocks and miniprep DNA have been made and are in lab.

Using the above SNase constructs and other lab constructs,

20 plasmids coding for LexA-SNase fusions have been engineered and transformed into *E. coli*; frozen stocks and miniprep DNA have been made and are in lab (strains [V770E, V776E]).

Snase clones were transformed into *Escherichia* coli and then

25 into *Saccharomyces* cerevisiae (yeast) (FY250). Yeast containing the SNase clone were grown and harvested, and SNase was purified using a Ni-affinity column. Purified SNase (single band on a Coomassie stained gel, see Figure 8) was analyzed using MS. The expected molecular weight for SNase is ~20,017 Da; a peak at

30 19,774 Da is likely from SNase. See Figure 9. The difference in expected molecular weight (244 Da) corresponds to the molecular weight of two amino acids (assuming amino acid average molecular weight to be 114 Da). This peak is very strong (relative to background) and is well resolved from other

35 signals.

These results demonstrate the use of MS to identify purified SNase. Further, this allows one to theorize that this approach may be successful in the detection and identification of TG-mediated post translational modification of a target protein (in this example SNase).

Subcloning of Microbial Transglutaminase (S.mobaraense) - Expression in Yeast and Activity Assays

In an effort to address the reasonable possibility that the TG substrate sequence on the SNase protein may function, function better, or function only when fused to the B42 activation domain (instead of the LexA DNA binding domain), B42 fusions were made as well. Plasmids coding for B42-SNase fusions have been constructed and transformed into E. coli; frozen stocks and miniprep DNA have been made and are in lab (strains [V762E, V769E].

Plasmid on which construct is based	Fusion protein	TG substrate sequence	Strain name (Bacteria/ TG1)	Strain name (Yeast/ FY251)
pEG202	LexA-SNase	LLQG +	V770E	See 80601*
pEG202	LexA-SNase	LQGG ++	NYM*	NYM**
pEG202	LexA-SNase	LGQG +++	NYM*	NYM**
pEG202	LexA-SNase	LGGG ++++	NYM*	NYM**
pJG4-5	B42-SNase	LLQG +	V762E	NYM**
pJG4-5	B42-SNase	LQGG ++	V794E	NYM**
pJG4-5	B42-SNase	LGQG +++	NYM*	NYM**
pJG4-5	B42-SNase	LGGG ++++	NYM*	NYM**

*Patches made but have not named strain nor made frozen stocks.
**NYM (not yet made)
+ (SEQ. ID. NO. : 3)
++ (SEQ. ID. NO. : 2)
+++ (SEQ. ID. NO. : 1)
++++ (SEQ ID. NO. : 4)

Three of the eight proposed constructs have been made (see Table) and tested. Based on the success wiht the three constructs made, the other constructs are as expected to work. Early constructs and experiments with those constructs were based on TG from both S. mobaraense and S. cinnamoneum. However, other are available.

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